REMARKS

Rejection of the Claims Under 35 U.S.C. §112, second paragraph

Claims 2-4, 13-15, and 24 stand rejected under §112, second paragraph for allegedly being indefinite by reciting the limitation "monoclonal antibodies". The Examiner is directed to the Supplemental Reply which was filed on May 29, 2002 wherein these claims were rewritten to recite "monoclonal antibody". This rejection is now moot and should be withdrawn.

Claims 3-4, 13-15, and 24 stand rejected for reciting the phrase "in this way". The claims have been rewritten to refer to the preceding step, as suggested by the Examiner. Therefore, this rejection is also moot and should be withdrawn.

Rejection of the Claims Under 35 U.S.C. § 103

Claims 1-4, 13-15 and 24 stand rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over June et al in view of Tacke et al. Applicants respectfully traverse for the reasons given below.

June et al. discloses a method for selectively stimulating proliferation of T cells by stimulating the T cell receptor (TCR)/CD3 complex or the CD2 surface protein. Proliferation is then induced with an accessory molecule, e.g, CD28 or CD9, which binds its cognate ligand. The primary activation signal actually requires the second, costimulatory signal (col. 5, lines 17-20). June discloses that various antibodies to CD28 have been reported including monoclonal antibodies: 9.3, KOLT-2, 15E8, 248.23.2 and EX5.3D10 (col. 5, lines 52-57). Careful review of the scientific literature which details the characteristics of these different antibodies reveals that these antibodies are not direct stimulatory antibodies of T cells.

Monoclonal (Mab) 9.3 is murine IgG2a antibody which is directed against the T cell CD28 receptor. June et al. (1990) (*Immunology Today*, 11:211-216) dislcoses that mAb9.3 stimulation of the CD28 receptor alone did not induce T-cell proliferation (see page 213, second paragraph) and makes reference to several technical papers which describe the effects of the binding of mAb 9.3 to the T cell. June et al. (1987) *Mol. Cell. Biology*, 7:4472-4481 discloses that "the binding of monoclonal antibodies to the CD28

antigen on purified T cells does not result in proliferation", see e.g. abstract of the paper. Additionally, Hara et al. (1985) *J. Exp. Med.* 161:1513-1524 discloses that "mAb 9.3 was not mitogenic either for PMS or isolated T cells. In the presence of TPA, mAb 9.3 was strongly mitogenic for T cells" (see e.g., p. 1516, third paragraph).

Experimental data from other technical papers further demonstrate the absence of T-cell activation by human T-cells with mAb 9.3 alone. For example in a proliferation time course assaying the stimulatory effects of mAb9.3 in combination with CD2 or CD3 mAb, proliferation was not detected when mAb 9.3 was added alone (see e.g. Figure 2 in Van Lier et al. (1988) *Eur. J. Immunol.*, 8:167-172). Also, T cells cultured in the presence of mAb 9.3 alone did not show any induction of an activation as measured by the increase
in IL-2 production or secretion (see e.g. Table III in Verwilghen et al. (1993) *J. Immunol.* 150: 835-846).

Monoclonal (mAb) Kolt-2 is a murine IgG1 antibody which is directed against the T cell CD28 receptor (June et al. (1990), *Immunology Today*, 11:211-216). The Kolt-2 mAb behaved similarly to the mAb 9.3 in T cell mitogenic assays. Moreover, the antibody by itself was not mitogenic but in combination with PMA, T-cell proliferation was observed (see e.g., Van Lier et al. (1988) *Eur. J. Immunol.*, 8:167-172 at page 168). Also, North et al. makes additional disclosure that mAb Kolt-2 does not have a stimulatory effect on the proliferation of purified T cells in normal donors or patients with CVID as measured by ^{3}H-thymidine incorporation (North et al. (1994) *Clin. Exp. Immunol.*, 95: 204-208, Figures 1 and 2). Denning et al. further discloses that "Kolt-2 alone had no effect upon thymocyte proliferation" (Denning et al. (1988) *J. Immunol.* 141: at page 2982, second paragraph).

Monoclonal (mAb) 15E-8 is a murine IgG1 antibody which is directed against the T cell CD28 receptor (June et al. (1990), *Immunology Today*, 11:211-216). Romano et al. discloses that anti-CD28 MAB CLB-CD28/1 clone 15E8 alone does not induce proliferation of PBMC from normal donors as determined from ^{3}H-thymidine incorporation (Romano et al. (1993) *Cellular Immunology*, 148:455-463, Figures 4 and 5). Another paper of Romano discloses that the 15E8 antibody alone does not induce IL-2 production by PBMC from normal donors (see e.g., Romano et al. (1994) *Cellular*

Immunology, 156:371-377 at Table 1). Los et al further discloses that an anti-CD28 mAb, termed LCB28, which is presumably MAB CLB-CD28/1 alone does not induce IL-2 production in human T cells (Los et al. (1995) *EMBO J.* 14:3731-3740).

Monoclonal (mAb) 248.23.2 is a murine IgM antibody which is directed against the T cell CD28 receptor (June et al. (1990), *Immunology Today*, 11:211-216). Pierres et al. provides further experimental evidence that human thymocytes or thymic subsets do not respond to anti-CD28 mAb 243 with proliferation as determined by ^{3}H-thymidine incorporation (Pierres et al. (1990) *J. Immunol.* 144, 1202-1207, see Tables III and IV).

Finally, the monoclonal antibody Ex5.3D10 is disclosed in June et al. A search of databases reveals no further disclosure about this antibody. This antibody was not available from the European ATCC as evidenced by the attached email regarding availability of the antibody. Moreover, an on-line inquiry into the US ATCC data base also reveals that the antibody is not available. The ATCC deposit number ((HB11373) recited in the June et al. patent at column 6, lines 7-9) for the EX5.3D10 antibody was searched and no entries were found (see the attached print-out of that search).

Thus, even if the Examiner were correct that motivation existed to screen the June et al. antibodies, the result would not be to arrive at an antibody of the instant invention. Thus, on this basis alone the rejection must be withdrawn. Furthermore, the references cited herein are part of the state of the art and must be considered herein in determining whether the necessary motivation exists. They clearly establish, for the reasons summarized above, that motivation did not exist to screen the June et al. antibodies as the Examiner suggests since the antibodies were known not to be directly stimulating.

Claims 1-4, 13-15 and 24 stand rejected under Seifken et al. The claims have been rewritten to recite that no artificial cross-linking between the antibody to the CD28 receptor with a secondary monoclonal antibody is required to activate human T cells. This rejection is now moot and should be withdrawn.

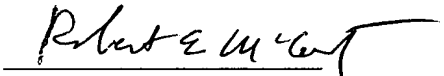
In view of the above remarks and amendments, it is submitted that this application is now ready for allowance. Early notice to this effect is solicited.

However, if there are any remaining issues which can be expeditiously resolved by a telephone conference, the Examiner is courteously requested to telephone the undersigned at the number indicated below.

The Commissioner is hereby authorized to charge any fees associated with this response or credit any overpayment to Deposit Account No. 13-3402.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached pages are captioned "Version With Markings to Show Changes Made".

Respectfully submitted,



Robert E. McCarthy, Reg. No. 46,044
Representative Capacity

Anthony J. Zelano, Reg. No. 27,969
MILLEN, WHITE, ZELANO & BRANIGAN, P.C.
Arlington Courthouse Plaza
2200 Clarendon Blvd., Suite 1400
Arlington, VA 22201
Direct Dial: (703) 812-5322
email: mccarthy@mwzb.com

Attorney Docket: Albre-20
Date filed: March 5, 2003

Version With Markings to Show Changes Made

Please **amend** the claims 1, 3-4, 13-15, and 24 as follows:

1. (Twice Amended) A human-compatible monoclonal antibody which is specific for human CD28 and activates human T-lymphocytes of several to all sub-groups without being artificially crosslinked with a secondary antibody and without occupancy of an antigen receptor of the human T-lymphocytes and thus antigen-non-specifically, and which is effective for treating a disease with pathologically reduced number of CD4 T-cells or an autoimmune disease.

3. (Twice Amended) A monoclonal antibody according to claim 1, with the hybridoma cells enabled to produce monoclonal human-CD28 specific animal antibodies being available through

a) creation of a plasmid by means of insertion of human-CD28 cDNA into the pHβAPr-1-neo vector following excision of the SalI-HindIII fragment and production of protoplasts from Escherichia coli (MC1061) which carry the plasmid,

b) fusing of the protoplasts with mouse A20J and/or L929 tumour cells using polyethylene glycol,

c) cultivation of the transfected cells received in b) above,

d) screening of the transfected mouse A20J and/or L929 cells for the expression of human CD28 and selection of mouse A20J and/or L929 cells expressing human-CD28,

e) immunization of BALB/c mice with mouse A20J and/or L929 cells expressing human-CD28,

f) removal of spleen cells of the mice immunized in this way and fusing the spleen cells with cells of the cell line X63-Ag 8.653 by means of polyethylene glycol to produce the hybridoma cells,

g) selection of the hybridoma cells ~~received in this way~~ produced in f) above with the condition that in the supernatant of selected hybrdoma cells there

are antibodies contained which bind on human CD28 expressing mouse A20J and/or L929 cells, and

h) cultivation/sub-cloning of the selected hybridoma cells obtained in g) above and isolating the monoclonal antibodies.

4. (Amended) A hybridoma cell for the production of a monoclonal antibody according to claim 1, which is available through the following:

a) creation of a plasmid by means of insertion of human-CD28 cDNA into the pHβAPr-1-neo vector following excision of the SalI-HindIII fragment and production of protoplasts from Escherichia coli (MC1061) which carry the plasmid,

b) fusing of the protoplasts with mouse A20J and/or L929 tumour cells using polyethylene glycol,

c) cultivation of the transfected cells received in b) above,

d) screening of the transfected mouse A20J and/or L929 cells for the expression of human CD28 and selection of mouse A20J and/or L929 cells expressing human-CD28,

e) immunization of BALB/c mice with mouse A20J and/or L929 cells expressing human-CD28,

f) removal of spleen cells of the mice immunized ~~in this way~~ as in e) above and fusing the spleen cells with cells of the cell line X63-Ag 8.653 by means of polyethylene glycol, and

g) selection of the hybridoma cells received in this way with the condition that in the supernatant of selected hybrdoma cells there are antibodies contained which bind on human CD28 expressing mouse A20J and/or L929 cells.

13. (Amended) A monoclonal antibody according to claim 2, enabled to produce monoclonal human-CD28 specific animal antibodies being available through

a) creation of a plasmid by means of insertion of human-CD28 cDNA into

the pHβAPr-1-neo vector following excision of the SalI-HindIII fragment and production of protoplasts from Escherichia coli (MC1061) which carry the plasmid,

b) fusing of the protoplasts with mouse A20J and/or L929 tumour cells using polyethylene glycol,

c) cultivation of the transfected cells received in b) above,

d) screening of the transfected mouse A20J and/or L929 cells for the expression of human CD28 and selection of mouse A20J and/or L929 cells expressing human-CD28,

e) immunization of BALB/c mice with mouse A20J and/or L929 cells expressing human-CD28,

f) removal of spleen cells of the mice immunized ~~in this way~~ as in e) above and fusing the spleen cells with cells of the cell line X63-Ag 8.653 by means of polyethylene glycol to produce the hybridoma cells,

g) selection of the hybridoma cells received ~~in this way~~ as in f) above with the condition that in the supernatant of selected hybridoma cells there are antibodies contained which bind human CD28 expressing mouse A20J and/or L929 cells, and

h) cultivation/sub-cloning of the selected hybridoma cells obtained in g) above and isolating of the antibodies therefrom.

14. (Amended) A hybridoma cell for the production of a monoclonal antibody according to claim 2 which is available through the following:

a) creation of a plasmid by means of insertion of human-CD28 cDNA into the pHβAPr-1-neo vector following excision of the SalI-HindIII fragment and production of protoplasts from Escherichia coli (MC1061) which carry the plasmid,

b) fusing the protoplasts with mouse A20J and/or L929 tumour cells using polyethylene glycol,

c) cultivation of the transfected cells received in b) above,

d) screening of the transfected mouse A20J and/or L929 cells for the expression of human CD28 and selection of mouse A20J and/or L929 cells expressing human-CD28,

e) immunization of BALB/c mice with mouse A20J and/or L929 cells expressing human-CD28,

f) removal of spleen cells of the mice immunized in this way and fusing the spleen cells with cells of the cell line X63-Ag 8.653 using polyethylene glycol, and

g) selection of the hybridoma cells received ~~in this way~~ as in e) above with the condition that in the supernatant of selected hybrdoma cells there are antibodies contained which bind human CD28 expressing mouse A20J and/or L929 cells.

15. (Amended) A hybridoma cell for the production of a monoclonal antibody according to claim 3 which is available through the following:

a) creation of a plasmid by means of insertion of human-CD28 cDNA into the pHβAPr-1-neo vector following excision of the SalI-HindIII fragment and production of protoplasts from Escherichia coli (MC1061) which carry the plasmid,

b) fusing of the protoplasts with mouse A20J and/or L929 tumour cells using polyethylene glycol,

c) cultivation of the transfected cells received in b) above,

d) screening of the transfected mouse A20J and/or L929 cells for the expression of human CD28 and selection of mouse A20J and/or L929 cells expressing human-CD28,

e) immunization of BALB/c mice with mouse A20J and/or L929 cells expressing human-CD28,

f) removal of spleen cells of the mice immunized ~~in this way~~ as in e) above and fusing the spleen cells with cells of the cell line X63-Ag 8.653 using polyethylene glycol, and

g) selection of the hybridoma cells received in this way with the condition that in the supernatant of selected hybrdoma cells there are antibodies contained which bind human CD28 expressing mouse A20J and/or L929 cells.

24. (Amended) A hybridoma cell for the production of a monoclonal antibody according to claim 13 which is available through the following:

a) creation of a plasmid by means of insertion of human-CD28 cDNA into the pHβAPr-1-neo vector following excision of the SalI-HindIII fragment and production of protoplasts from Escherichia coli (MC1061) which carry the plasmid,

b) fusing of the protoplasts with mouse A20J and/or L929 tumour cells using polyethylene glycol,

c) cultivation of the transfected cells received in b) above,

d) screening of the transfected mouse A20J and/or L929 cells for the expression of human CD28 and selection of mouse A20J and/or L929 cells expressing human-CD28,

e) immunization of BALB/c mice with mouse A20J and/or L929 cells expressing human-CD28,

f) removal of spleen cells of the mice immunized ~~in this way~~ as in e) and fusing the spleen cells with cells of the cell line X63-Ag 8.653 using polyethylene glycol, and

g) selection of the hybridoma cells received in this way with the condition that in the supernatant of selected hybrdoma cells there are antibodies contained which bind human CD28 expressing mouse A20J and/or L929 cells.